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13. ABSTRACT ***(Maximum 200 Words)***

Preclinical studies with passively administered monoclonal antibodies or vaccine induced antibodies against glycolipid and mucin antigens have protected mice from tumor recurrence, even when treatment was initiated after tumor challenge. This timing is comparable to the adjuvant setting in the clinic. The glycolipid LeY and mucin MUC1 are expressed at the cell surface of most breast cancer cells in over 80% of breast cancer biopsy specimens. The optimal approach for antibody induction against these antigens has been conjugation to the immunogenic carrier molecule KLH and mixture with the potent immunological adjuvant QS21. The LeY-KLH and MUC1-KLH plus QS-21 vaccines prepared and tested over the last year have resulted in high titer antibodies against the synthetic antigens which were of relatively modest titer against tumor cells expressing these antigens. While these vaccines could be included in future polyvalent vaccines, it is our impression that we can augment the relevant immunogenicity by the modifications proposed which should make the immunogens more closely resemble the natural antigens. Consequently, glycosylated MUC1 peptides and LeY clusters will be conjugated to KLH, mixed with QS21, and tested over the next year.

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FOREWORD

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INTRODUCTION

Due to the 75% reduction in funding level from our original grant application the work scope has been restricted to the production and pre-clinical testing of MUC1 and Lewis Y vaccines for patients with breast cancer or ovarian cancer. The goal of the trials is to induce antibodies against MUC1 and Lewis Y which are cell surface antigens broadly expressed on cancers of the ovary and breast. Initial clinical trials with both preparations have been conducted over the last year and preliminary results are available. Modified version of these two vaccines (second generation) are currently being prepared for testing.

BODY

MUC1

We had previously immunized breast cancer patients with a MUC1-KLH (Keyhole Limpet Hemocyanin) plus QS-21 adjuvant vaccine containing 1 ½ repeats of the MUC1 20aa tandem repeat. This 32aa MUC1 vaccine induced high titer antibodies against MUC1 in essentially all immunized patients but these antibodies reacted weakly with the cell surface of tumor cells expressing MUC1 (1). Consequently, a variety of modifications of the MUC1 peptide have been identified for testing and this represents the first such trial. A 106aa MUC1 peptide expressing more than 5 repeats of the 20aa tandem repeat was prepared. This is no simple feat. This long peptide was prepared with a terminal cystine for linkage to KLH. Since the conjugation efficiency is only 15%, 30mg of the MUC1 peptide were required. The peptide was purified to exclude shorter MUC1 peptides, sequenced to confirm the proper sequence and conjugated to KLH using an M-maleimidobenzoyl-N-hydroxy succinimide (MBS) as previously described. Unbound MUC1 was excluded with a 30,000 molecular weight filter and the conjugate mixed with QS-21 and vialed. The epitope ratio of MUC1 to KLH was 560 to 1. Vials were opened to confirm sterility, purity, safety and immunogenicity as required by the FDA. Thirteen breast cancer patients were treated with this preparation. The vaccine was well tolerated with local erythema and induration lasting 2-4 days experienced by all patients and occasional low grade flu-like symptoms or fever last 12-24 hours experienced by occasional patients. This is categorized as grade 1 systemic and grade 2 local toxicity. No unexpected toxicities were encountered. Patients received five immunizations over a four-month period, receiving the initial three immunizations at one-week intervals. Pre and peek post immunization ELISA titers against purified MUC1, pre and post flow cytometry results against MCF7 are demonstrated in the table below.

Table 1
SUMMARY OF SEROLOGICAL RESPONSE TO VACCINATION WITH MUC1 (106AA)-KLH+QS21

Patient	No. of Vaccination	Peak ELISA titer IgM Pre	Peak ELISA titer IgG Post	IgM FACS % Positive Cells Pre	IgM FACS % Positive Cells Post	IgM FACS % Positive Cells Pre	IgM FACS % Positive Cells Post
1	5	0	10	0	0	10.5	19
2	5	0	5120	0	320	10	41
3	5	0	640	0	320	9.2	22
4	5	0	160	0	640	10.3	10.4
5	5	0	640	0	5120	10.4	71
6	5	0	2560	0	5120	10.5	44
7	3	0	320	0	320	9.6	29
8	5	0	320	0	320	10	32
9	5	0	1280	0	320	10.3	46
10	5	0	1280	0	2560	10.2	70
11	5	0	640	0	640	9.4	14
12	5	0	320	0	640	10.4	17
13	5	0	2560	0	1280	9.6	16

It was anticipated that the longer MUC1 sequence would permit the peptide to assume a more physiologic tertiary configuration and hence result in the induction of antibodies more able to react with the tumor cell surface. This was not the case. As demonstrated with our previous shorter MUC1 peptides, high titer antibodies by ELISA were induced in most patients but these reacted only weekly with the tumor cell surface. We conclude from this that the longer peptide was no better that the shorter peptide and since it was far more laborious and expensive to prepare, no further studies with the MUC1 106aa peptide vaccine will be conducted. Consequently, we have focused on the other major possibility for augmenting the cell surface reactivity of vaccine induced antibodies against MUC1 as it is expressed at the cell surface. This involves the use of a glycosylated MUC1 peptide. This has been achieved through collaboration with Dr. Henrik Clausen of the Netherlands and the use of the T2 and T4 glycosyl transferases. We have prepared a 106aa MUC1 peptide fully glycosylated with N-acetyl galactose at all five sites per tandem repeat and a 32aa MUC1 glycosylated at three of the five potential sites. These have been conjugated to KLH and we are in the process of vialing vaccines for preclinical testing.

Lewis Y (Le^Y)

Lewis Y pentasaccaride was synthesized as the allyl glycoside as described previously. It was conjugated to KLH following reductive amination with an Le^Y-KLH conjugate ratio of 310-1. The yield of conjugated Le^Y in this reaction was 8%. Le^Y-KLH conjugate was vialed at four different concentrations with QS-

21 and the vials tested for sterility, safety, and immunogenicity. Twenty-four patients were vaccinated with vaccines containing 3, 10, 30 or 60mg of Le^Y in-groups of six patients (2). The peak titer IgM and IgG ELISA results against Le^Y and the pre and post immunization flow cytometry results at the four different vaccine doses are demonstrated in the table below. The 10μg dose was selected for testing in future vaccination trials. However, the ELISA titers and flow cytometry results were not as striking as initially hoped and so a second generation Le^Y vaccine containing Le^Y clusters is being prepared. This would contain three Le^Y pentasaccarides linked to sequential or alternating serines on a short peptide chain with a terminal cystine, which is used for linkage to KLH. Synthesis of these clustered Le^Y molecules is currently in progress.

TABLE 2

Summary of Serological Responses to Vaccination with Le^Y-KLH+QS21

Vaccine Le^Y Dose	No of Patients	Peak Median ELISA Titer IgM	IgG	Median Peak FACS % Positive Cells	Median CDC % Lysis
3μg	6	20	0	10	7.3
10μg	6	80	0	26	29
30μg	6	40	0	24	19
60μg	6	20	0	8.6	7

KEY RESEARCH ACCOMPLISHMENTS

1) Preparation of a 106aa MUC1 peptide with proper sequence for conjugation to KLH and vaccine production.

2) Preparation of the MUC1-KLH vaccine and completion of pre-clinical and clinical testing.

3) Synthesis of LeY pentasaccarides for vaccine production.

4) Preparation of the LeY conjugate vaccine and completion of pre-clinical and clinical testing.

5) Preparation of second generation MUC1 and LeY vaccines containing glycosylated MUC1 and LeY clusters.

REPORTABLE OUTCOMES

Two manuscripts have been submitted. The references are below.

1. Gilewski T., Adluri, S., Zhang, S., Ragupathi, G., Houghton, A., Norton, L. and Livingston, P.O. Vaccination of high risk breast cancer patients with Mucin-1 keyhole limpet hemocyanin conjugate plus QS-21. Clin Can Res, Submitted.

2. Sabbatini, P., Kudryashov, V., Danishefsky, S., Livingston, P.O., Ragupathi, G., Bornmann, W., Spassova, M., Spriggs, D., Aghajanian, C., Soignet, S., Peyton, M., O'Flaherty, C., Curtin, J. and Lloyd, K.O. Immunization of ovarian cancer patients with a synthetic LewisY – protein conjugate vaccine: clinical and serological results. Int J. Cancer. Submitted.

CONCLUSIONS

The MUC1 and LeY vaccines prepared and tested over the last year have resulted in high titer antibodies against the synthetic antigens which were of relatively modest titer against tumor cells expressing these antigens. While these vaccines could be included in future polyvalent vaccines, it is our impression that we can augment the relevant immunogenicity by the modifications proposed. Consequently, glycosylated MUC1 peptides and LeY clusters will be tested over the next year.

REFERENCES

1. Gilewski T., Adluri, S., Zhang, S., Ragupathi, G., Houghton, A., Norton, L. and Livingston, P.O. Vaccination of high risk breast cancer patients with Mucin-1 keyhole limpet hemocyanin conjugate plus QS-21. Clin Can Res, Submitted.

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